Amendments to the Specification

Please amend Paragraphs [0006], [0009], [0032], [0034], [0040], [0043], [0045], [0056], [0058], [0061], [0062], [0063] and [0074], as designated in the application as filed, or Paragraphs [0008], [0011], [0034], [0036], [0042], [0045], [0047], [0058], [0060], [0063], [0064], [0065] and [0076], as designated in the application as published (Publication No. 20040142484), as follows:

[0006] FIG. 1 shows various steps used ~~a method~~ for identifying a sample based on a resonance enhanced spectroscopic analysis, according to embodiments of the invention.

[0009] FIG. 4 shows Stokes Raman spectra for dilute aqueous solutions of four DNA nucleotides~~, according to embodiments of the invention~~.

[0032] FIG. 1 shows some of the steps used ~~a method~~ for identifying a sample based on a resonance enhanced stimulated Raman spectroscopic analysis, according to embodiments of the invention. The steps outline ~~method allows~~ identifying a sample based on spectroscopic data that serves as a fingerprint or signature for the sample. In brief, the steps include ~~method includes~~ adding a sample, such as a single molecule of interest in solution, to a resonant spectroscopic analysis chamber at block 110, analyzing the sample with a resonance enhanced stimulated Raman spectroscopy at blocks 120-160, and identifying the sample based on the analysis at block 170. In some embodiments of the invention, the resonance enhanced stimulated Raman spectroscopy may include irradiating a sample contained in a resonance chamber at block 120, scattering radiation from the sample at block 130, resonating the scattered radiation in the chamber at block 140, ~~irradiating or~~ transmitting the scattered radiation from the chamber at block 150, and detecting the irradiated scattered radiation at block 160. In one aspect, the steps ~~method~~ may be used in coordination with nucleic acid sequencing and may include identifying a

single nucleic acid derivative in a sample received from a nucleic acid sequencing system in an effort to sequence a DNA or RNA molecule.

[0034] Initially, with reference to block 110 ~~of the method 100~~, the sample 230, which may include a liquid, gas, or mixed fluid, is added to the chamber 250. In the system illustrated in FIG. 2, the sample is added to the chamber by flowing through the fluid channel. The channel is a void or hollowed-out space within a solid material and may be, for example, any tube, pipe, duct, or conduit to convey a fluid. The cross section of the channel may have a circular, oval, square, rectangular, or other shape. The fluid channel has a sample inlet 275 that is coupled with a sample source to receive a sample for analysis and a sample outlet 280 that is coupled with a sample destination to discharge an analyzed sample. The sample may be received at the inlet, flowed through the channel, flowed into the chamber, analyzed, subsequently flowed out of the chamber, and flowed through the outlet to the proper destination. Flow may be achieved by providing an appropriate driving force, such as a pressure differential between the inlet and the outlet or a pump within the channel. As one example, ~~an~~ a diluted aqueous solution of a ~~diluted~~ nucleic acid derivative may be received from a pressurized nucleic acid sequencing system by pressure injection and added to the chamber by flowing through the channel in order to identify the particular nucleic acid derivative. Alternatively, the sample may be added with a fluid pump, such as a syringe, through an opening to the chamber, rather than by flowing through a fluid channel, and subsequently removed with the syringe. In still other implementations, if the sample is electrically charged it may be added to the chamber under the force provided by an electric field, or if the sample is magnetic (e.g., has a magnetic moiety attached) it may be added to the chamber by the force provided by a magnetic field.

[0040] Once the sample is contained within the chamber, with reference to block 120 ~~of the method 100~~ (FIG. 1), the sample is irradiated. In the system illustrated in FIG. 2, the source 210 provides input radiation 215 to the chamber and irradiates the sample positioned therein. Suitable

sources include coherent light sources, lasers, light emitting diodes (LEDs), lamps, and fiber optic cables coupled with such a source of radiation. Radiation sources that provide strong monochromatic or quasimonochromatic radiation will often be favored over those that provide weaker or broad-spectrum radiation, since such monochromatic radiation facilitates detection of the relevant offset spectroscopic signals. The source 210 may contain filters or monochromators to reduce certain frequencies. The source may also contain lenses, mirrors, or other devices to redirect and focus the radiation on the chamber. These devices are commercially available from numerous sources, including vendors that are listed in "The Photonics Directory.TM.: The Photonics Buyers' Guide To Products and Manufacturers". This directory contains vendor listings for radiation sources, radiation detectors, spectrometers, spectroscopic analysis software, as well as numerous other accessories (e.g., lenses, wavelength selection devices, etc.). The Photonics Directory.TM. is available from Laurin Publishing, and is presently available online at the website ~~website~~: www.photonics.com/directory/index.asp.

[0043] As previously discussed, the transmitted input radiation 225 is introduced into the cavity and some of the input radiation, or some of the reflected input radiation, or both, irradiates the sample. With reference to block 130 ~~of the method 100~~ of FIG. 1, some of the radiation is scattered by the sample. As used herein, the terms "scattered radiation" and the like will be used to refer to a photon of light or other radiation that has collided with and been absorbed by sample matter, such as a molecule, and has been released or emitted. Most of the scattered photons will be released elastically in which the scattered radiation has no change of energy or frequency. This radiation is known as Rayleigh scattered radiation. Some of the scattered photons, often a small fraction, will be released inelastically in which there is an exchange of energy and a change of frequency. These are well-known phenomenon. Stokes scattered radiation refers to scattering where the molecule loses energy and the frequency is reduced whereas anti-Stokes scattered radiation refers to scattering where the molecule gains energy and the frequency is increased. This scattering of light by matter is known as Raman spectroscopy. FIG. 3

conceptually illustrates Rayleigh, Stokes, and anti-Stokes radiation. Stokes and anti-Stokes radiation typically have lower intensity than Rayleigh radiation, and respectively exist at lower and higher frequencies than Rayleigh radiation. As used herein, Stokes and anti-Stokes radiation will be referred to collectively as inelastically scattered radiation.

[0045] As previously discussed, it is often difficult and improbable to detect, or reliably detect, spontaneously scattered radiation from a dilute molecule in solution. Accordingly, with reference to block 140 ~~of the method 100~~ of FIG. 1, the present inventors have discovered systems and methods for resonating radiation in a cavity in order to perform a resonance enhanced spectroscopic analysis of a sample. The resonated signal is stronger and easier to detect than the spontaneous signal. Advantageously, this may allow improved probability and reliability of accurately and reliably detecting and identifying dilute molecules of interest in solution.

[0056] With continued reference to ~~the method 100 of~~ FIG. 1, and to block 150, after resonating, the scattered radiation may be irradiated or transmitted from the chamber. Referring to FIG. 2, the chamber may contain at least one window to allow the radiation to exit the chamber and be detected. The particular chamber 250 contains an outlet window 260, which in this particular instance comprises the partial reflector 245. The partial reflector is an incomplete or finite reflector that has a sufficient reflectivity to achieve resonance and a sufficient transmittance to allow a detectable level of incident radiation to be transmitted to the detector 290. The reflector 235 may have a higher total reflectance than the partial reflector 245 in order to provide good amplification of intensity in the cavity, although this is not required. In one instance, the reflector 235 may have a high reflectivity for both the input excitation radiation and the inelastically scattered radiation, for example greater than approximately 99% (e.g., approximately 99.9%) for both, whereas the partial reflector 245 may have a sufficient transmittance for the inelastically scattered radiation, for example a transmittance that is not less than approximately 1% (or a reflectance that is not greater than approximately 99%). Of course alternate types of outlet

windows may also be used, such as a transparent material incorporated into the cavity housing, a Pockel cell, a space, gap, slit, pinhole, or other opening in the reflector 235, or a shutter. Additionally, as another alternative, a single inlet-outlet window may be utilized to transmit radiation into and out of the chamber.

[0058] With reference again to ~~the method 100 of~~ FIG. 1, at least some of the inelastically scattered radiation in the output radiation 285 is detected at block 160. The electromagnetic radiation detector 290 is shown in simplified format and is to be interpreted broadly. Often, the output radiation may be passed through a lens and a wavelength selection device. The lens may collect, direct, and focus the radiation emitted from the chamber. The light from the lens or from the chamber may be passed through a wavelength selection device that emphasizes, selects, separates, or isolates a particular frequency or range of frequencies. The wavelength selector may be used to distinguish radiation of interest (e.g., inelastically scattered radiation) from other radiation (e.g., elastically scattered radiation and excitation radiation). Suitable wavelength selection devices include among others prisms, monochromators, filters (e.g., absorbance, bandpass, interference, or Fourier), dichroic filters, dichroic mirrors, and demultiplexers.

[0061] Often, the spectrometer may generate spectra showing frequency or wavelength versus intensity. FIG. 4 shows Stokes Raman spectra for highly diluted aqueous samples of each of the four DNA nucleotides~~, according to embodiments of the invention~~. For clarity, the four spectra have been offset or displaced from one another along the vertical axis. From top to bottom, the spectra are for deoxyadenosine monophosphate, deoxycytidine monophosphate, deoxyguanosine monophosphate, and deoxythymidine monophosphate. The spectra were generated by performing spontaneous Raman spectroscopic analysis on the samples including irradiating the samples with 514 nanometer radiation from a laser and detecting the output inelastically scattered radiation with a Raman spectrometer. The spontaneous spectra should be substantially similar to resonance enhanced Raman spectra except that the signals would be correspondingly

weaker in intensity. The figure shows intensity in arbitrary units versus Raman wavelength shift (Stokes offset from the excitation radiation) in reciprocal centimeters. As shown, each of the spectra have distinct spectral characteristics, prominent peaks, or fingerprint bands, that identify the corresponding nucleotide. Exemplary prominent peaks include 1630 cm^{-1} for the top spectra, 1440 cm^{-1} for the next spectra down, 800 cm^{-1} for the second spectra from the bottom, and 1350 cm^{-1} for the bottom spectra.

[0062] Finally, with reference to ~~the method 100 of~~ FIG. 1, the sample may be identified at block 170. The sample may be identified by comparing the determined spectrum with a spectral library containing many predetermined spectrum for known samples and identifying the sample as one of the known samples if the determined spectrum sufficiently approximates the corresponding predetermined spectrum in the library. The electrical signals generated by the radiation detector as a result of the output radiation may be provided to a computer system that is appropriately programmed with spectroscopic analysis instructions and the library (e.g., a database) that allows the Raman fingerprint or signature represented in the electrical signals to be correlated to a specific fingerprint or signature in the library. For example, in the case of a spectrometer, a spectrum (or certain bands thereof) for a sample may be compared or contrasted to spectroscopic data for other identified samples to determine whether the fingerprints are sufficiently identical (i.e., the sample has the same identity as the identified sample in the database). Various methods for identification of nucleotides by Raman spectroscopy are known in the art (see e.g., U.S. Pat. Nos. 5,306,403, 6,002,471, or 6,174,677). The identity of the sample may be stored in memory, further analyzed, or used for other purposes.

[0063] With reference again to ~~the method 100 of~~ FIG. 1, ~~another way of implementing this method~~ the steps may include adding a sample to a chamber at block 110, irradiating the sample with a short and accurately-known pulse of a radiation having substantially the same frequency or wavelength as an inelastically scattered wavelength relevant to the sample at block 120, then

scattering, resonating, and irradiating the radiation from the cavity at blocks 130-150, respectively, and then detecting the radiation as well as some indication of the decay time of the inelastically scattered light in the chamber at block 160. The decay time may be used as a fingerprint or signature for the identity of the sample. Of course other methods are also contemplated.

[0074] Accordingly, with continued reference to ~~the method 100 of~~ FIG. 1, irradiating a sample at block 110 may include irradiating a sample with an input transverse radiation and an input seed radiation having different frequencies. Likewise, detecting radiation at block 160 may include detecting a gain over the intensity of the seed radiation. The seed radiation frequency may be varied over a set of predetermined frequencies corresponding to different molecules to determine whether the sample within the chamber contains a molecule corresponding to one of these frequencies. For example, the seed radiation may be provided at an inelastically scattering frequency for adenine. If the gain over the seed radiation is sufficiently zero then there was no simulated scattering and it may be concluded that the sample did not contain adenine. Then the frequency of the seed radiation may be adjusted to that corresponding to other nucleotide bases until the gain is sufficiently nonzero indicating that the sample may contain that particular base. If none of the frequencies cause the gain to become sufficiently nonzero, the sample may be removed from the chamber and another sample added thereto.